Change in Substrate Binding Specificity of the Tandem Acyl Carrier Protein Domains of Polyunsaturated Fatty Acid Synthesis

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Background

Polyunsaturated fatty acids synthases are large enzymatic complexes that produce PUFAs, such as eicosenoic and docosahexaenoic (DHA, 22:6ω3). These ω-3 fatty acids are essential for health and nutrition. In general, ω-3 fatty acids are found in fish, some unicellular organisms like marine GammaProteobacteria, and Myxobacteria. The bacterial PUFAs synthases are analogous to polyketide synthases (PKS), which are involved in the bacterial antibiotic production. Understanding the enzymology and biochemistry of PUFAs synthases continues to be rudimentary.

Results & Discussion

As described in the literature, ACPs can be self-acylated with malonyl-CoA but not with acetyl-CoA. Similarly, the KSAT domain promotes the binding of malonyl-CoA to the ACPs but not acetyl-CoA. However, the opposite is observed in the active site mutant AT-S703A. We propose that some other polar residue could be performing the role of AT-S703A. Also, we wanted to optimize the observation method of the mutants of AT domains.

Hypothesis

Molecular weight of the KS-AT domain of PfaA. The KS-AT domain has a molecular mass of 130 kDa.

Methodology

1. Protein Overexpression and Purification.

2. Gel Electrophoresis of ACPs

3. Radioactivity Assays

4. Constructions of One-Point Mutations

5. Purification of AT-S703A

6. AT-S703A Radioactivity Assay

Conclusions

- It was determined that ACPs acylate with malonyl-CoA but not acetyl-CoA. We have proved that KS-AT promotes the binding of malonyl-CoA to the ACPs but not acetyl-CoA.
- When the serine active site of the AT domain is mutated (AT-S703A), the AT domain transfers an acetyl-CoA residue to the ACPs instead of malonyl-CoA.
- This change in substrate binding specificity presents a new result to understand the specificity of AT domains.

References